THE PURIFICATION OF MALIC DEHYDROGENASE FROM RAT LIVER

BY

BIMAL KUMAR BACHHAWAT

B. S., St. Xavier's College, 1946

M. S., University College of Science and Technology, 1948

R. UNIV. BIBLIOTHEEK LEIDEN

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS

1953

THE PURIFICATION OF MALIC DEHYDROGENASE FROM RAT LIVER

BY

BIMAL KUMAR BACHHAWAT

B. S., St. Xavier's College, 1946

M. S., University College of Science and Technology, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1953

URBANA, ILLINOIS

1953

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. C. S. Vestling for his invaluable advice and friendly interest throughout the course of these investigations and to Dr. D. M. Gibson for his helpful advice.

The author also wishes to thank Mr. R. L. Fischer who kindly performed the electrophoretic and radioactive zinc analyses, Miss Mary L. Killey for her cooperation in developing the transaminase assay, Mr. H. Crespi for performing the ultracentrifugal analysis, and Mrs. Patricia Potts for her help in analyses.

THE PURIFICATION OF MALIC DEHYDROGENASE FROM RAT LIVER

Introduction

The important role of malic acid as an intermediate in the oxidative metabolism of two carbon fragments has been well established. Malic dehydrogenase (MDH), which catalyses the interconversion of the malate-oxalacetate system, thus plays an important part in the Krebs citric acid cycle. Moreover, the interrelationship of this enzyme with the malic enzyme and glutamate-aspartate transaminase, and its role in CO_2 fixation with oxalacetate decarboxylase stimulated our interest in the fractionation of MDH. As a part of a study of the nature of the proteins of liver, which contains what Edsall (1) has referred to as "an incredible multiplicity" of enzymes, we chose this organ as a source of MDH.

Discussion of Results

Extraction of MDH from liver homogenates with a low ionic strength-alcohol mixture has been successful. This partial extraction technique (2) lead to two and a half times the purification of a high ionic strength extraction. The residue obtained after low ionic strength extraction can be reextracted with a higher ionic strength medium. In this reextracted liquid we have usually about 90 per cent of the lactic dehydrogenase. Thus by the use of a partial extraction it has been shown that we can extract two different enzymes. By modifying the zinc precipitating technique of Cohn *et al* (3) it has been shown that zinc ion can effectively separate hemoglobin from MDH. The novel technique of determining zinc by the use of radioactive Zn^{65} as a tracer helped us to follow the distribution of zinc in various fractions. We have shown with the aid of this technique that the initial zinc-protein interaction is greatly affected by the pH of the medium. With the help of Zn^{65} we were able to show that the complete removal of zinc from proteins can be achieved by the use of "metal buffers" and dialysis. During the purification of MDH it was observed that MDH was highly

unstable on dialysis. However, in the earlier fractionation procedure we were able to minimize denaturation by the use of a concentrated protein solution and rapid dialysis. The purified fraction (200 fold) as obtained was light yellow in color, and this fraction on dialysis suffered about ⅓ denaturation. Although this dialysed fraction gave an almost symmetrical pattern in the ultracentrifuge, the electrophoretic pattern indicated that the fraction has suffered partial denaturation on dialysis. The sedimentation value of MDH indicates that MDH is a low molecular weight protein. MDH has been further characterized by determining its K_M value for DPN and malate.

From the results of the studies on the effect of sulfhydryl reagents on MDH it can be safely concluded that MDH contains important sulfhydryl groups. This is further confirmed by the reversal of inhibition by cysteine. That sulfhydryl groups are involved in enzyme-substrate complex formation was indicated by the fact that the preincubation of MDH with either of the substrates results in partial protection of MDH from PCMB inhibition.

During the studies on the effect of pH changes on MDH activity it was observed that the optimum pH is around 10. With the help of versene buffer it has been also shown that MDH most probably does not require any multivalent metallic ion for its activity.

From the results of the specificity study for MDH it has been shown that MDH is DPN specific. This DPN specificity for MDH is contrary to the earlier reports on crude pigeon liver MDH (4).

A new assay for transaminase which is 10-15 times more sensitive than the older method (5) has been devised by coupling the transaminase reaction with the MDH-catalyzed reduction of oxalacetate by DPNH. The reproducibility of the result is very encouraging. Moreover, the simplicity and the accuracy of the method suggests that this assay will be more useful than other methods reported earlier.

Summary

1) The distribution of MDH in rat liver mitochondria, micro-

somes and cytoplasm has been studied.

2) With the help of a partial extraction technique, zinc chloride precipitation, a "dialysing in" technique and acetone fractionation at low ionic strength, a highly active MDH fraction has been obtained.

3) With the help of Zn^{65} as a tool, the distribution and the removal of zinc during the fractionation have been studied.

4) The ultracentrifugal and electrophoretic study of the partially purified fraction has been reported.

5) The effect of sulfhydryl reagents and changes in pH on MDH have been studied.

6) The specificity of the enzyme has been investigated.

7) A new spectrophotometric assay for glutamate-aspartate transaminase has been developed.

BIBLIOGRAPHY

1. J. T. Edsall, *Enzymes and enzyme systems* (Harvard University Press, Cambridge, Mass. 1951), p. xi.
2. E. J. Cohn, D. M. Surgenor and M. J. Hunter, *Enzymes and enzyme systems* (Harvard University Press, Cambridge, Mass. 1951), p. 15.
3. E. J. Cohn, *Blood cells and plasma proteins* (Academic Press Inc., New York, 1953), p. 28.
4. A. R. Mehler, A. Kornberg, S. Grisolia and S. Ochoa, *J. Biol. Chem.*, **164**, 961 (1948).
5. P. S. Camarata and P. P. Cohen, *J. Biol. Chem.*, **193**, 45 (1951)

VITA

The author was born on August 16, 1925 in Calcutta, India. He received his Bachelor of Science degree from St. Xavier's College, Calcutta, in 1946. In 1948 he received a Master of Science degree in Applied Chemistry from the University College of Science Technology, Calcutta. Then he joined the department of Food Technology, Jadavpur College of Engineering, Calcutta, as a research assistant. In January, 1950 he came to this country and joined the Division of Antibiotics, Food and Drug Administration, Washington, D. C., as a research trainee. In the fall of that year he enrolled in the Graduate College of the University of Illinois and held a research assistantship for one year and a third year University Fellowship.